Characterization of The Prognostic m6A-Related lncRNA Signature For Predicting Immune Responses In Esophageal Cancer

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Primary research

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Abstract

Background: This study aimed to construct a long non-coding RNAs (lncRNAs) signature related to N6-methyladenosine, so as to precisely forecast the prognosis of esophageal cancer patients.

Methods: In this study, we downloaded RNA inference data and test data of patients with Esophageal cancer from the TCGA database, and passed skin-related tests, single-variation coxs and selection operator (LASSO) Cox regression to all subjects related to the subtypes and risks. The results were analyzed for survival and predicted with different properties and values of risk models. Finally, we also discussed the potential immunotherapeutic characteristics and drug sensitivity prediction of the model.

Results: Firstly, 93 lncRNAs related to prognosis were obtained through co-expression and univariate Cox analysis. Then we also performed least absolute shrinkage and selection operator (LASSO) analysis to obtain AC012467.2, AL161891.1, ARHGAP27P1-BPTFP1-KPNA2P3, LINC00930, POLR2J4, AP003696.1, AP003696.1 and MAGI1-AS1 to construct m6A-related lncRNA model. The subsequent consensus clustering analysis divided these samples into two parts. We observed that the survival curves of patients from different clusters and risk groups were significantly different. With the help of Kaplan-Meier survival analysis and ROC curve, we confirmed the difference of survival rates among different risk groups, and proved the prognostic value of risk signals. We observed that there were significant differences in the characteristics of immunotherapy between high-risk and low-risk groups, including the immune cell infiltration, immune gene expression, immunosuppressive points, and drug sensitivity.

Conclusions: m6A-related lncRNAs prognostic signature was shaped that probably functions as vital mediators of tumor microenvironment of esophageal cancer, and might become underlying biomarkers to forecast the prognosis of esophageal cancer patients.

Background

Esophageal cancer (ESCA) is a terrifically invasive malignancy, ranking the eighth among the most popular malignant tumor worldwide. In addition, the mortality rate of esophageal cancer remains high, ranking sixth in the world, and has been soaring in recent years [1; 2; 3]. In the statistics of National Central Cancer Registry of China (NCCR) in 2015, Chinese ESCA patients account for up to 70% of all ESCA cases all over the world[4].

At present, most esophageal cancer patients necessitated extensive treatment, including chemotherapy, chemoradiotherapy and/or esophagectomy [5]. Although surgical resection combined with radiotherapy and chemotherapy improves the prognosis of ESCA patients, the overall 5-year survival rate is still very low [6].

Therefore, elucidating the molecular mechanism of esophageal cancer and identifying new molecular targets are very important for its diagnosis and treatment.

N6-methyladenosine (m⁶A), the amplest inside modification of RNA in eukaryotic cells, has attracted growing notice last several years. Accumulating data has shown that m⁶A RNA methylation plays a fundamental role in diagnosis, treatment and prognosis of cancer patients by regulating circadian cadence, gene manifestation, stress reaction, inflammatory behavior, and carcinogenesis [7; 8; 9; 10]. Researchers had realized that m⁶A methylation has great potential in the pathogenesis and treatment of diseases [11; 12; 13; 14]. Recently, many studies began to pay attention to the function of m⁶A modification in regulating the occurrence and advancement of esophageal cancer [15; 16; 17; 18]. For example, it was found that HNRNPA2B1, one of m⁶A readers, facilitates esophageal cancer succession by regulating ACLY and ACC1. Bioinformatics found that the expression of HNRNPA2B1 in ESCA went up. In cell experiments, HNRNPA2B1 promoted fatty acid synthesis, cell transportation and infringement in ESCA [19].

Previous studies have reported the reliability of abnormal lncRNA expression as a diagnostic and prognostic marker of tumors. However, the role of lncRNAs in m⁶A modification in esophageal cancer has not been reported.

In this study, we identified m⁶A related lncRNAs using Pearson correlation analysis and constructed a prognostic feature of m⁶A related lncRNAs. Then this is further verified in test data set and train data set. Subsequently, we explored the relationship between this prognostic model and the tumor microenvironment in a variety of ways. Finally, using the publicly available drug sensitivity database, we found candidate drugs for this m⁶A related lncRNAs.

Materials And Methods

Authentication of m⁶A-associated lncRNAs

We have downloaded the RNA transcriptome dataset and the correspondent **ESCA** clinic-related information from The Cancer Genome Atlas (TCGA) (<https://portal.gdc.cancer.gov/>) database. The RNA-seq transcriptome data were standardized by fragment per kilobase of exon model per million. The clinicopathological information of patients included survival status, survival time, age, gender and tumor lymph node metastasis (TNM) stage.

Subsequently, we have assembled genes into protein coding genes and lncRNA genes in view of the human genome annotation data. Besides, given the previous publications. we extracted expression matrixes of 23 m⁶A RNA methylation regulators (Additional file2) from the TCGA databases based on the recent the m⁶A study. The pearson correlation coefficient has been utilized to assess the relationship between m⁶A regulator genes and lncRNA. The lncRNA with an utter correlation coefficient >0.3 and a P value <0.05 was regarded as a m⁶A-associated lncRNA.

Construction of a model of m⁶A related lncRNA related to prognosis

After extracting the m⁶A related lncRNA, the ESCA cohort was congregated into separated groups by consistent cluster analysis using "ConsensusClusterPlus" in R[20]. The ESCA cohort was congregated into various parts by non-negative matrix factorization (NMF) clustering approaches. Overall survival (OS) differences were calculated by Kaplan Meier method and log rank test were used to calculate the overall survival differences. Through the analysis of univariate Cox, the relationship between m⁶A regulators and OS of ESCA patients has been appraised via survival analysis in R. The research has further analyzed m⁶A genes and advanced a risk signature by employing the least absolute shrinkage and selection operator (LASSO) Cox regression algorithm. The formula of the risk score for ESCA patients' prognosis prediction was as follows: Risk score = $\sum \text{Expi} * \beta_i$. where Expi refers to each lncRNA expression and β_i equals to the coefficient of each lncRNA. Based on the median risk score, the patients have been equivalently separated into high- and low-risk subgroups. Each patient's survival status, death time, and gene expression profile in two subgroups were presented via "heatmap" and "survival" R packages. Among that, we have conducted the Kaplan–Meier curve research, and the receiver operating characteristic (ROC) curve was drawn to evaluate the sensitivity and specificity of the prognostic signature.

Analysis Of Immune Related Aspects

ESTIMATE(<https://sourceforge.net/projects/estimateproject/>) is widely used to figure out the matrix score and immunized score in malignant tumor tissues. We performed correlation analysis in different groups. Subsequently, the degree of infiltration of immune cells was quantified by Single Sample Gene Set Enrichment Analysis (ssGSEA). In addition, tumor related biomarkers were selected from previous studies for comparison between subtypes. Then, Kruskal Kallis test was performed. The mRNA expression based RNAss and DNA methylation based DNAss were used to measure tumor stemness [88]. Then we screened out genes that are important for immune cell regulation (Additional file3), the "ggplot2", "ggpubr" and "ggextra" packages in R were utilized to analyze the relationships between gene expression and the risk scores, and the disparity in expression levels between patients at high- and low-risk of m⁶a related lncRNA. In the subsequent drug sensitivity analysis, we used the contents of cellminer website and listed the top 16 drugs with the most closely correlation.

Results

Identification of m⁶A-associated lncRNAs in patients with ESCA

The matrix expression of 23 m⁶A regulatory gene and 14087 lncRNAs was extracted from TCGA database. Among that, 491 lncRNAs was found significantly linked with m⁶A-related genes by pearson correlation analysis, and the co-expression network has been displayed in Additional file1. The expression data has been secured from TCGA and the survival time and survival state of the modified lncRNAs have

been well combined with m⁶a. These lncRNAs have been analyzed by univariate Cox analyses (figure1A), and we found that 93 lncRNAs were closely pertaining to the total survival rate. The results have indicated that ARHGAP27P1-BPTFP1-KPNA2P3 and EPB41L4A-DT constituted protective genes, but other genes were high-risk genes and their expression was closely entwined with poor prognosis. Finally, we discovered that the expression of the lncRNAs was greatly different between ESCA patients and healthy controls by utilizing heatmap (figure1B).

Consensus Clustering of m⁶A-modified lncRNAs

When the clustering index “k” increased from 2–9, the interference between clusters was minimal when k=2. Meanwhile, K = 2 is proved to be the optimal clustering parameter between clusters (figure2A,2B). The survival time of patients and the expression level of the selected lncRNAs have been organically combined. Ultimately, ESCA patients were secured and classified into two clusters, cluster 1 and cluster 2, on the basis of the expression of the m⁶A-modified lncRNAs (figure2C). Survival analysis based on the lncRNA subtypes was employed to appraise the prognostic value of m⁶A-lncRNAs, and the survival rate of cluster 1 was tested to be higher than that of cluster 2 (p = 0.007), as demonstrated in Figure 2D. Heatmap outlines the differences that were recognized in the expression of prognosis-associated lncRNAs and the correlation has been appraised based on clinicopathological parameters (Figure 2E). After that, we compared the clinicopathological characteristics of the two clusters and their correlation were tested, then we found that the tumour node metastases (TNM) stage, age, and gender had no straight correlation with our cluster analysis (Figure 2F).

As detecting the proportion of 22 immune cell subtypes between cluster 1 and cluster 2 (figure2H), we have observed that cluster 2 was equipped with higher follicular helper T cells infiltration (figure2F), while neutrophils expressed more in cluster 2 (figure2G).

Construction and validation of prognostic signatures for m⁶a-modified lncRNAs

To shape a risk model to evaluate the premonitory risk of patients with ESCA. ESCA patients have been divided into two groups, train cohort and test cohort. Then we investigated expression levels of 93 m⁶A modified lncRNAs by a least absolute shrinkage and selection operator (LASSO) regression analysis (figure3A, 3B), and eight critical lncRNAs related to m⁶A and their coefficients were identified. These eight lncRNAs were independently connected to OS, including AC012467.2, AL161891.1, ARHGAP27P1-BPTFP1-KPNA2P3, LINC00930, POLR2J4, AC007128.1, AP003696.1 and MAGI1-AS1. Risk score = $AC012467.2 \times 0.11 + AL161891.1 \times 0.02 + ARHGAP27P1-BPTFP1-KPNA2P3 \times (-0.08) + LINC00930 \times 0.20 + POLR2J4 \times 0.34 + AC007128.1 \times 0.12 + AP003696.1 \times 0.98 + MAGI1-AS1 \times 0.03$.

The survival analysis illustrated that the OS of the low-risk group was longer than that of the high-risk group despite of whether patients were in the train ($p = 0.004$) (figure4A) or test group ($p = 0.003$) (figure4E). In an attempt to deeply determine the sensitivity and specificity of the risk signatures for diagnosis, we have also applied a ROC curve with the AUC values for the risk signatures being 0.937 and 0.729, respectively, in the train and the test cohorts (figure4B,4F).

We categorized patients in the ESCA train and test groups into low- and high-risk ones by considering the median value of the prognostic risk grade. The allocation of survival and death between the low-risk and high-risk groups is described in figure5A and figure5D, and the survival status and survival time of patients in the two separated risk groups are displayed in figure5B and figure5E. We observed that the prognosis of the low-risk group was better than that of the high-risk group. The related expression of the 8 m⁶A-associated lncRNAs for each patient are demonstrated in figure5C and figure5F. The results of heat map showed that most genes were highly expressed in the high-risk group.

The congregation capacity of the m⁶A-associated lncRNA model could be better confirmed through principal component analysis (PCA). It was initiated to examine the disparity between the low-risk and high-risk groups grounded on the complete gene manifestation profiles, 23 m⁶A genes, all m⁶A-related lncRNAs, and risk genes based on LASSO analyses (Figures 6A–6D). Figures 6A to 6C present that the allocation of the high- and low-risk groups has been distributed to some extent. Nonetheless, the research outcome secured on the basis of our model has also demonstrated that the low- and high-risk groups had different distributions (Figure 6D). These results suggest that the prognostic signature can distinguish between the low- and high-risk groups.

Independent prognostic role of the lncRNA signature

A univariate cox regression analysis and multivariate cox regression analysis have been performed between the risk score and the clinical factors in the train and test groups to ascertain the independence of lncRNA signature in the clinicopathological features (like age, gender, TNM stage and risk score. As shown in Figures 3C,3D,3G and 3H, it can be discovered that the risk score can function as an independent prognostic factor for the prognosis of ESCA to some extent.

The OS disparities in age, gender, stage and TNM stage between high-risk group and low-risk group have been further ascertained, so as to confirm the practicability of our model in different clinical groups. We observed that in addition to female, M1, Stage III-IV, N1-3 and T3-4 groups, other groups' OS in low-risk groups were higher than that in high-risk group (FIGURE 7), which demonstrated that our model was meaningful to a large extent.

Similarly, we compared the clinical characteristics between the high-risk and low-risk group and detect their relationship. We have discovered that there remained no straight connection between the age and stage (figure3A). Interestingly, we observed that there remained a certain correlation between risk score classification and cluster classification, and Cluster 2 is relevant to a higher risk score (figure3B).

Analysis of immune-related factors in two risk subgroups

According to the ImmuneScore, and StromalScore generated by the ESTIMATE algorithm, the StromalScore were to a large extent, negatively relevant to the risk score ($P < 0.01$), as shown in Figure 8A.

Studies have shown that abnormal DNA methylation may lead to poor prognosis of cancer patients [42]. Our analysis found that DNAss, which was based on DNA methylation, was positively correlated with risk scores (figure 8B). This means that the higher the degree of DNA methylation of esophageal cancer cells, the worse the prognosis of patients. Additionally, ssGSEA analysis showed that the ratio of B cells and NK cells have been greatly raised in the low-risk subgroup (figure 8C). In the meantime, we also found that the expression of cytolytic activity and HLAs was high in the low-risk group (figure 8D).

After meticulously investigating the connection between the risk score and several notable HLA manifesting levels in the TCGA database, we have seen that the manifestation of almost all HLA genes (HLA-A, HLA-DRB6, HLA-F, HLA-E, HLA-B and HLA-H) were up-modulated in the LOW-risk group (figure9A,9C-9G). Through correlation analysis, we found that HLA genes were negatively correlated with the risk scores (figure9B), suggesting that HLA specific treatment could be of more practicability for patients in low-risk groups.

we have also explored the disparity in immune signatures between the two risk subgroups.

We downloaded immune-associated genes, then the manifestation differences of these immune-associated genes in high and low risk groups were compared. The finding stated that there were significant disparities in the manifestation of 12 immune related genes between high-risk group and low risk group (figure10A). We then dug out the correlation between these genes and risk scores and their expression in high and low risk groups. Interestingly, we observed that TNFSF8, B2M, VSIR, PPF1, HAVCR1 and TLR3 were highly exhibited in the low-risk group and negatively correlated with the risk score. HSP90B1, HSPA1A, HSPA14, BLRC5, IFNA21 and EZH2 were intensely mirrored in high-risk group, which was positively correlated with risk score (figure10B-10M).

Drug Sensitivity Analysis

We selected the top 16 drugs with the strongest correlation, and observed that the correlation between three lncRNAs and drug sensitivity was the strongest, namely LINC00930, MAGI1-AS and POLR2J4 (figure11). We can observe that the manifestation of these genes is positively linked to the sensitivity of most drugs, that is, the higher the expression of these genes in patients with esophageal cancer, the more useful these drugs are for the treatment of esophageal cancer. Interestingly, we found that LINC00930, MAGI1-AS and POLR2J4 were tremendously expressed in the high-risk group, indicating that the drugs were more effective in the treatment of patients in the high-risk group.

Discussion

A large number of lncRNAs are highly modified by m⁶A to achieve their biogenesis and function [21; 22]. In tumor research, we observed that lncRNA interacted with m⁶A affected cancer progress, metastasis, drug resistance and immune response[23]. lncRNA KIAA1429 actually serves as a powerful engine for liver cancer progression through N6-methyladenosine-dependent post-transcriptional modification of GATA3[24]. m⁶A methylation, serving as a good agent to stabilize LNCAROD, could also advance cancer progression by shaping a ternary complex with HSPA1A and YBX1 in head and neck squamous cell carcinoma[25]. Although the function of lncRNAs and m⁶A in the regulation of tumor development have been clarified in multitudinous authoritative, there remained unknown on the lncRNAs in the regulation of m⁶A in esophageal cancer.

According to pearson correlation coefficient, we have discovered an intimate relationship between m⁶A methylation modulators and m⁶A-modified lncRNAs. Based on the expression of m⁶A-modified lncRNAs, the ESCA patients were divided into two clusters by uniform clustering. Compared with cluster 1, the survival rate of cluster 2 was lower, and it was no straight link with other clinical characteristics.

After that, we have appraised the prognostic value of the m⁶A-modified lncRNA signatures in ESCA patients, employed the LASSO algorithm, and selected 8 lncRNAs to ascertain the risk signature. As the formula shows, the risk value of each sample has been figured out. The results of KM curve analysis showed that this feature can effectively stratify the OS of patients and has robust prognostic value. Further ROC analysis showed that the characteristic risk score had a good prognosis and might be an effective biomarker of esophageal cancer.

The staging of TNM by the international American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) provides classification guidelines for the degree of cancer spread. According to the evaluation of various cancers by the staging system, the progression and invasion of tumors during surgical resection and the prognosis of patients were evaluated. However, there are significant differences in clinical outcomes in the same histological tumor stage, which indicates that the TNM staging system has some limitations.

Up to now, many methods have been proposed to expand cancer classification, but most methods mask the heterogeneity within the tumor and ignore the role of tumor microenvironment.

Here, we not only analyzed the reliability of m⁶A related lncRNA as a prognostic biomarker, but also focused on the immune determinants affecting cancer development, such as immunoscore, which can be prognostic and predictive biomarkers.

"Immunoscore" is an immune based assay used to quantify in situ T cell infiltration. At present, immunoscore is most widely used in predicting OS in colon cancer. Studies have proved that immunoscore's prediction of the prognosis of stage I, II and III colon cancer exceeds the traditional TNM system. In stage I, II or III colon cancer, patients with high immunoscore have the lower risk of recurrence[26]. Our results confirmed that the immunoscore in the low-risk group was higher, which was

negatively correlated with the risk score. In the prognostic analysis of esophageal cancer, we speculated that the specific immune environment characteristics and high immune score in esophageal cancer may be predictors of better prognosis and longer long-term survival.

In addition, we also focused on the differences of previously neglected immune cells in high-risk and low-risk groups, such as NK cells and B cells.

It could be seen from the study that the soaring of B cell number was closely entwined with better clinical outcome, such as hepatocellular carcinoma[27], non-small cell lung cancer[28], and aggressive ductal breast carcinoma[29].

NK cells had strong capacity to impede tumor growth and regulate the initial stages of metastatic dissemination [30; 31]. The occurrence of innate and adaptive immune responses and tumor immunosurveillance have been significantly attributed to NK cells by identifying and strangling neoplastic cells [32; 33]. Multiple enduring clinic-related trials contrive to activate the immune system with NK cell treatment. For instance, NK cell therapy in hematologic malignancies is an effective strategy [34].

Infiltrating NK cells and B cells may become an important evidence to evaluate the prognosis of esophageal cancer in the future.

HLAs are gradually considered as an immune checkpoint for cancer due to its immunosuppressive function[35]. For example, studies have confirmed that HLA-G expression is associated with tumor metastasis and adverse clinical outcomes[36; 37]. In addition, some studies have pointed out that HLA is very important for the interaction between tumor cells and innate and adaptive immune cells, like NK cells and T cells[38].

Through cautiously observing the association between risk scores and HLA expression levels, we have also discovered that most HLA (HLA-DPB2, HLA-DQA2, HLA-DQB2, HLA-DRB6 AND HLA-K) were expressed in the low-risk group. Therefore, human leukocyte antigen is very likely to become a potential target of new immunotherapy and a new immune checkpoint inhibitor. However, our results also suggest that HLA as a new immune checkpoint inhibitor may not be suitable for all esophageal cancer cells. On the contrary it may be more suitable for low-risk esophageal cancer with better prognosis.

In spite of the above major findings of the study, there still exist some limitations that should be amended or reconsidered in current research. Firstly, the limited number of TCGA databases leads to a small number of ESCA samples in our research. In the future, we need more samples to verify this result. Secondly, this time, we mainly apply the bioinformatics method. Although this method has many advantages in the current research, it still needs more basic experiments to prove the results of this study. Third, because the current team of researchers still lacks a sample bank of human esophageal cancer, it is necessary to establish a sample bank of esophageal cancer tissue in the future.

Conclusion

Our results reveal the underlying biomarkers and therapeutic targets of the risk model grounded on m⁶A-related lncRNAs. It will enrich the research on the m⁶A-related lncRNAs in esophageal cancer, which deserves in-depth exploration by follow-up researchers.

Declarations

Availability of data and materials

A publicly available database was analyzed in this study; it can be found in the The Cancer Genome Atlas (<https://portal.gdc.cancer.gov/>).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Qin Zhang: Conceptualization, Methodology, Writing- Original draft preparation, Luyan Zhang: Supervision, Visualization, Software

References

- [1] B. Gupta, and N. Kumar, Worldwide incidence, mortality and time trends for cancer of the oesophagus. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP)* 26 (2017) 107-118.
- [2] R.L. Siegel, K.D. Miller, and A. Jemal, Cancer statistics, 2018. *CA: a cancer journal for clinicians* 68 (2018) 7-30.
- [3] E.C. Smyth, J. Lagergren, R.C. Fitzgerald, F. Lordick, M.A. Shah, P. Lagergren, and D. Cunningham, Oesophageal cancer. *Nature reviews. Disease primers* 3 (2017) 17048.
- [4] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, F. Bray, A. Jemal, X.Q. Yu, and J. He, Cancer statistics in China, 2015. *CA: a cancer journal for clinicians* 66 (2016) 115-32.
- [5] T. Lerut, and O. Wiesel, History of esophagectomy for cancer of the esophagus and the gastroesophageal junction. *Annals of translational medicine* 9 (2021) 897.

- [6] P.S.N. van Rossum, N.H. Mohammad, F.P. Vleggaar, and R. van Hillegersberg, Treatment for unresectable or metastatic oesophageal cancer: current evidence and trends. *Nature reviews. Gastroenterology & hepatology* 15 (2018) 235-249.
- [7] Y. Fu, D. Dominissini, G. Rechavi, and C. He, Gene expression regulation mediated through reversible m⁶A RNA methylation. *Nature reviews. Genetics* 15 (2014) 293-306.
- [8] T. Sun, R. Wu, and L. Ming, The role of m⁶A RNA methylation in cancer. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 112 (2019) 108613.
- [9] Q. Lan, P.Y. Liu, J. Haase, J.L. Bell, S. Hüttelmaier, and T. Liu, The Critical Role of RNA m⁶A Methylation in Cancer. *Cancer research* 79 (2019) 1285-1292.
- [10] R. Karthiya, and P. Khandelia, m⁶A RNA Methylation: Ramifications for Gene Expression and Human Health. *Molecular biotechnology* 62 (2020) 467-484.
- [11] C. Zhang, J. Fu, and Y. Zhou, A Review in Research Progress Concerning m⁶A Methylation and Immunoregulation. *Frontiers in immunology* 10 (2019) 922.
- [12] Y. Pan, P. Ma, Y. Liu, W. Li, and Y. Shu, Multiple functions of m⁶A RNA methylation in cancer. *Journal of hematology & oncology* 11 (2018) 48.
- [13] L. He, H. Li, A. Wu, Y. Peng, G. Shu, and G. Yin, Functions of N⁶-methyladenosine and its role in cancer. *Molecular cancer* 18 (2019) 176.
- [14] T. Wang, S. Kong, M. Tao, and S. Ju, The potential role of RNA N⁶-methyladenosine in Cancer progression. *Molecular cancer* 19 (2020) 88.
- [15] L.C. Xu, J.X. Pan, and H.D. Pan, Construction and Validation of an m⁶A RNA Methylation Regulators-Based Prognostic Signature for Esophageal Cancer. *Cancer management and research* 12 (2020) 5385-5394.
- [16] H. Hou, H. Zhao, X. Yu, P. Cong, Y. Zhou, Y. Jiang, and Y. Cheng, METTL3 promotes the proliferation and invasion of esophageal cancer cells partly through AKT signaling pathway. *Pathology, research and practice* 216 (2020) 153087.
- [17] W. Guo, F. Tan, Q. Huai, Z. Wang, F. Shao, G. Zhang, Z. Yang, R. Li, Q. Xue, S. Gao, and J. He, Comprehensive Analysis of PD-L1 Expression, Immune Infiltrates, and m⁶A RNA Methylation Regulators in Esophageal Squamous Cell Carcinoma. *Frontiers in immunology* 12 (2021) 669750.
- [18] T.L. Xia, S.M. Yan, L. Yuan, and M.S. Zeng, Upregulation of METTL3 Expression Predicts Poor Prognosis in Patients with Esophageal Squamous Cell Carcinoma. *Cancer management and research* 12 (2020) 5729-5737.

- [19] H. Guo, B. Wang, K. Xu, L. Nie, Y. Fu, Z. Wang, Q. Wang, S. Wang, and X. Zou, m(6)A Reader HNRNPA2B1 Promotes Esophageal Cancer Progression via Up-Regulation of ACLY and ACC1. *Frontiers in oncology* 10 (2020) 553045.
- [20] M.D. Wilkerson, and D.N. Hayes, ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. *Bioinformatics (Oxford, England)* 26 (2010) 1572-3.
- [21] Y.C. Yi, X.Y. Chen, J. Zhang, and J.S. Zhu, Novel insights into the interplay between m(6)A modification and noncoding RNAs in cancer. *Molecular cancer* 19 (2020) 121.
- [22] X. Yang, S. Zhang, C. He, P. Xue, L. Zhang, Z. He, L. Zang, B. Feng, J. Sun, and M. Zheng, METTL14 suppresses proliferation and metastasis of colorectal cancer by down-regulating oncogenic long non-coding RNA XIST. *Molecular cancer* 19 (2020) 46.
- [23] S. Dinescu, S. Ignat, A.D. Lazar, C. Constantin, M. Neagu, and M. Costache, Epitranscriptomic Signatures in lncRNAs and Their Possible Roles in Cancer. *Genes* 10 (2019).
- [24] T. Lan, H. Li, D. Zhang, L. Xu, H. Liu, X. Hao, X. Yan, H. Liao, X. Chen, K. Xie, J. Li, M. Liao, J. Huang, K. Yuan, Y. Zeng, and H. Wu, KIAA1429 contributes to liver cancer progression through N6-methyladenosine-dependent post-transcriptional modification of GATA3. *Molecular cancer* 18 (2019) 186.
- [25] Y. Ban, P. Tan, J. Cai, J. Li, M. Hu, Y. Zhou, Y. Mei, Y. Tan, X. Li, Z. Zeng, W. Xiong, G. Li, X. Li, M. Yi, and B. Xiang, LNCAROD is stabilized by m6A methylation and promotes cancer progression via forming a ternary complex with HSPA1A and YBX1 in head and neck squamous cell carcinoma. *Molecular oncology* 14 (2020) 1282-1296.
- [26] F. Pagès, B. Mlecnik, F. Marliot, G. Bindea, F.S. Ou, C. Bifulco, A. Lugli, I. Zlobec, T.T. Rau, M.D. Berger, I.D. Nagtegaal, E. Vink-Börger, A. Hartmann, C. Geppert, J. Kolwelter, S. Merkel, R. Grützmann, M. Van den Eynde, A. Jouret-Mourin, A. Kartheuser, D. Léonard, C. Remue, J.Y. Wang, P. Bavi, M.H.A. Roehrl, P.S. Ohashi, L.T. Nguyen, S. Han, H.L. MacGregor, S. Hafezi-Bakhtiari, B.G. Wouters, G.V. Masucci, E.K. Andersson, E. Zavadova, M. Vocka, J. Spacek, L. Petruzella, B. Konopasek, P. Dundr, H. Skalova, K. Nemejcova, G. Botti, F. Tatangelo, P. Delrio, G. Ciliberto, M. Maio, L. Laghi, F. Grizzi, T. Fredriksen, B. Buttard, M. Angelova, A. Vasaturo, P. Maby, S.E. Church, H.K. Angell, L. Lafontaine, D. Bruni, C. El Sissy, N. Haicheur, A. Kirilovsky, A. Berger, C. Lagorce, J.P. Meyers, C. Paustian, Z. Feng, C. Ballesteros-Merino, J. Dijkstra, C. van de Water, S. van Lent-van Vliet, N. Knijn, A.M. Muşină, D.V. Scripcariu, B. Popivanova, M. Xu, T. Fujita, S. Hazama, N. Suzuki, H. Nagano, K. Okuno, T. Torigoe, N. Sato, T. Furuhashi, I. Takemasa, K. Itoh, P.S. Patel, H.H. Vora, B. Shah, J.B. Patel, K.N. Rajvik, S.J. Pandya, S.N. Shukla, Y. Wang, G. Zhang, Y. Kawakami, F.M. Marincola, P.A. Ascierto, D.J. Sargent, B.A. Fox, and J. Galon, International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet (London, England)* 391 (2018) 2128-2139.
- [27] Y. Wada, O. Nakashima, R. Kutami, O. Yamamoto, and M. Kojiro, Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. *Hepatology (Baltimore, Md.)* 27 (1998) 407-14.

- [28] K.I. Al-Shibli, T. Donnem, S. Al-Saad, M. Persson, R.M. Bremnes, and L.T. Busund, Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* 14 (2008) 5220-7.
- [29] Y. Xu, S. Lan, and Q. Zheng, Prognostic significance of infiltrating immune cell subtypes in invasive ductal carcinoma of the breast. *Tumori* 104 (2018) 196-201.
- [30] N. Shimasaki, A. Jain, and D. Campana, NK cells for cancer immunotherapy. *Nature reviews. Drug discovery* 19 (2020) 200-218.
- [31] S.Y. Wu, T. Fu, Y.Z. Jiang, and Z.M. Shao, Natural killer cells in cancer biology and therapy. *Molecular cancer* 19 (2020) 120.
- [32] E. Montaldo, G. Del Zotto, M. Della Chiesa, M.C. Mingari, A. Moretta, A. De Maria, and L. Moretta, Human NK cell receptors/markers: a tool to analyze NK cell development, subsets and function. *Cytometry. Part A : the journal of the International Society for Analytical Cytology* 83 (2013) 702-13.
- [33] K.L. O'Brien, and D.K. Finlay, Immunometabolism and natural killer cell responses. *Nature reviews. Immunology* 19 (2019) 282-290.
- [34] R.S. Mehta, B. Randolph, M. Daher, and K. Rezvani, NK cell therapy for hematologic malignancies. *International journal of hematology* 107 (2018) 262-270.
- [35] E.D. Carosella, N. Rouas-Freiss, D. Tronik-Le Roux, P. Moreau, and J. LeMaoult, HLA-G: An Immune Checkpoint Molecule. *Advances in immunology* 127 (2015) 33-144.
- [36] A. Lin, and W.H. Yan, Human Leukocyte Antigen-G (HLA-G) Expression in Cancers: Roles in Immune Evasion, Metastasis and Target for Therapy. *Molecular medicine (Cambridge, Mass.)* 21 (2015) 782-791.
- [37] W.H. Yan, Human leukocyte antigen-G in cancer: are they clinically relevant? *Cancer letters* 311 (2011) 123-30.
- [38] P. Leone, V. De Re, A. Vacca, F. Dammacco, and V. Racanelli, Cancer treatment and the KIR-HLA system: an overview. *Clinical and experimental medicine* 17 (2017) 419-429.

Supplementary Figure

Supplementary Figure 1 is not available with this version.

Figures

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Figure 1

Extraction of key m6A-Related lncRNA (A) Univariate Cox regression analysis revealed that the selected m6A-Related lncRNA significantly correlated with clinical prognosis (B) Heatmap of significantly m6A-Related lncRNAs. Red represents higher expression while green represents lower expression.

Image not available with this version

Figure 2

Identification of molecular subtypes in the complete dataset. (A) The cumulative distribution function (CDF) when k ranges from 2 to 9. (B) Area under CDF curve when k ranges from 2 to 9. (C) Consensus heatmap for the gene expression when k = 2. (D) Kaplan-Meier survival curves for the cluster when k = 2. (E) The heatmap showed the distribution of clinicopathological factors in two molecular subtypes. (F) The scores of follicular helper T cells infiltration and are shown in the box plot (G) The scores of neutrophils infiltration and are shown in the box plot. (H) The scores of 22 immune cells infiltration are shown in the box plot.

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Figure 3

Characterization of m6A-related lncRNAs signature (A,B) LASSO regression was performed, calculating the minimum criteria of the prognostic lncRNAs (C) The heatmap showed the distribution of clinicopathological factors and hub m6A methyltransferase-related lncRNAs between the high- and low-risk groups. (D) molecular subtypes differences between high and low risk groups.

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Figure 4

Prognostic analysis and Independent predictive ability in the training dataset and testing dataset (A, E) Kaplan-Meier curve analysis between the high-risk group and low-risk group was performed in the training dataset (A) and testing dataset (E), respectively. (B, F) The receiver operator curve analysis for the m6A-related lncRNAs signature in the training dataset (B) and testing dataset (F), respectively. (C, G) Validation of the independence of the m6A-related lncRNA signature in OS through the Univariate cox regression analysis in the training dataset (C) and testing dataset (G), respectively. (D, H) Validation of the independence of the m6A-related lncRNA signature in OS through the Multivariate cox regression analysis in the training dataset (D) and testing dataset (H), respectively.

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Figure 5

Prognostic value of the risk model of the 8 m6A-related lncRNAs In the training dataset and testing dataset (A , B) Different patterns of survival status and survival time between the high- and low-risk groups In the training dataset (A) and testing dataset (B) (C, D) Distribution of m6A-related lncRNA model-based risk score in the training dataset (C) and testing dataset (D) (E,F) Clustering analysis heatmap shows the expression standards of the 8 prognostic lncRNAs for each patient in the training dataset (E) and testing dataset (F).

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Figure 6

Principal component analysis between the high- and low-risk groups based on entire gene expression profiles(A), m6A-related lncRNAs(B), 23 m6A genes(C) and risk model based on the representation profiles of the 8 m6A-related lncRNAs(D) in the TCGA entire set.

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Figure 7

Kaplan-Meier survival curves for the high-risk and low-risk groups stratified by clinical factors including age, gender and TNM stage in the complete dataset.

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Figure 8

Analysis of immune function in two risk groups (A) Correlation analysis between risk score and DNAss and RNAss (B) Correlation analysis of risk score and ImmuneScore and StromalScore (C) The scores of 16 immune cells and are shown in the box plot (D) The scores of 13 immune-related functions are shown in the box plot

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Figure 9

Analysis of HLAs in high and low risk groups (A) The differential HLA expression was expressed by box plot (B-G) The differences in the relationship between HLAs and risk scores, as well as the relationship between HLAs and each other

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Figure 10

Top 16 drug sensitivity analysis

Supplementary Files

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